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Applicant(s): HOWARD L. WEINER *et al.*

Serial No.: 08/279,275 Examiner: P. Achutamurthy

Filed: July 22, 1994 Group Art Unit: 1818

For: **TREATMENT OF AUTOIMMUNE DISEASES BY ORAL ADMINISTRATION OF AUTOANTIGENS**

SECOND DECLARATION OF HOWARD L. WEINER

I, HOWARD L. WEINER, do hereby declare:

1. I hold a M.D. degree, conferred by the University of Colorado in 1969.

2. I am currently the Robert L. Kroc Chair ~~Professor~~ of Neurologic Disease at Harvard Medical School, and have held this position since 1985. I am also appointed as Physician in Medicine (Neurology) at Brigham & Women's Hospital, Boston, MA, and have held this appointment since 1987. Since 1985, I have been Co-Director of the Center for Neurologic Diseases at the same hospital. I am also a co-inventor named on the patent application indicated above. A copy of my *curriculum vitae* is attached.

3. I have extensive experience in the immunology of autoimmune diseases including in particular the oral use of antigens in the treatment of such diseases.

4. I understand that the Examiner at the U.S. Patent and Trademark Office who is handling this application has issued a rejection of claims on the basis of "lack of enablement". I have been informed that the factual basis underlying this rejection is the proposition that the present invention does not broadly apply to treatment of T-cell mediated autoimmune disease.

5. I have also been informed that the Examiner might find evidence persuasive in determining that the invention is generally applicable to T-cell mediated autoimmune disease which describes a shared mechanism of treatment according to this invention.

6. In fact, oral tolerance, the mechanism underlying the present invention, is a broadly applicable phenomenon that applies to ingestion of antigens generally. Below, I explain the oral tolerance mechanism and also briefly review treatments that have been developed that rely on this mechanism.

7. The immune system is the major biological defense mechanism responsible for recognizing and fighting disease. The immune system distinguishes foreign substances (antigens) from the body's own tissues and eliminates a wide variety of pathogenic agents such as bacteria and viruses. T cells, specialized white cells which circulate in the blood, are a major component of this system. Once a foreign substance, bacterium or virus, succeeds in penetrating

the physical barrier of the skin or mucosal membranes it encounters the immune system. In the gut mucosa, fragments of digested proteins or phagocytized bacteria are presented to resident T cells by Antigen-Presenting Cells (macrophages), resulting in T cell activation. There are several types of T cells, each of which plays a critical role in recognizing antigens, carrying out the immune response, or regulating the resulting chain of events. These include "helper" T cells, which release factors to amplify the immune response; "killer" T cells, which attack and destroy the cells displaying the targeted antigen; and "regulatory" T cells, which release factors to down-regulate or suppress the immune response and keep it from going out of control.

8. In normal individuals, T cells with specificity for self-antigens are either eliminated during differentiation or "suppressed" by regulatory mechanisms. In cell-mediated autoimmune disease, the T cells reactive for a self-tissue antigen become active and destroy healthy tissues and organs. The ultimate result is loss of organ functionality. Examples of cell-mediated autoimmune diseases include: Type I diabetes, where the pancreas is attacked with resultant loss of insulin production; multiple sclerosis, where brain or spinal cord tissue is attacked with a resultant loss of central nervous system (CNS) function; and, rheumatoid arthritis, where the cartilage in the joints is attacked and the resultant inflammation leads to joint destruction.

9. The method of the invention employs oral tolerance to interrupt and suppress the autoimmune disease process. It does so by stimulating the natural mucosal immune mechanisms associated with the small intestine, specifically the gut associated lymphoid tissues

(GALT). Tissue-specific autoimmune disease suppression can be achieved by orally delivering an appropriate amount of an autoantigen. These antigens are associated with the tissue under attack in an autoimmune disease. This type of therapy can be employed to treat a variety of T-cell mediated diseases and conditions.

10. The immune system is normally programmed to respond to foreign proteins encountered within the body by generating an inflammatory response which involves stimulation of Th1 helper cells. These T cells then release inflammatory cytokines such as IL-2 and IFN gamma. Entry of proteins through the mucosa, by contrast, stimulates a bias toward induction of TGF-beta secreting cells and Th2 cells which secrete IL-4 and IL-10, which results in a suppressive regulatory response.

11. The present invention treats human T-cell mediated autoimmune disorders by selectively suppressing the inflammatory immune responses associated with the disorder. It does so through oral administration of an autoantigen.

12. In fact, studies have demonstrated that not only do the autoantigens of the present invention function in this manner, but that tissue associated antigens in general suppress disease in autoimmune conditions in a disease-specific fashion when orally administered. With respect to the relevant animal disease models, for example, experimental autoimmune encephalomyelitis (EAE) is suppressed by oral treatment with myelin basic protein (MBP) and/or proteolipid protein (PLP); uveitis is suppressed by treatment with S antigen; myasthenia gravis is suppressed by treatment with acetylcholine receptor protein; collagen-, adjuvant-, Pristane-

and antigen-induced arthritis are suppressed by treatment with collagen; and diabetes is suppressed in non-obese diabetic (NOD) mice by treatment with insulin.

13. The success of these treatments using tissue associated antigens has led to clinical trials of several of these treatments in humans. Evidence has already been presented (see my first Declaration, filed May 30, 1993) of the clinical trials that have been undertaken for the treatment of multiple sclerosis by oral administration of bovine myelin, treatment of uveitis by oral administration of S-antigen, treatment of rheumatoid arthritis by oral administration of type II collagen, and treatment of type I diabetes by oral administration of insulin.

14. The use of oral tolerance to treat disease has also been demonstrated in animal models of tissue rejection. See, for example, the attached copy of U.S. Patent No. 5,593,698. As a result of these demonstrations, clinical trials have been initiated using oral administration of transplant antigens to suppress immune response in transplant rejection. Although these treatments do not use an "autoantigen", they tend to demonstrate the breadth of treatment that is possible using oral tolerance, i.e., the mechanism to which the present invention relates.

15. In treatments encompassed by the claims of this application, proteins are administered orally and broken down to fragments by the normal digestive processes. Specific fragments of these proteins (i.e., peptides) are taken up by antigen-presenting cells on the surface of the gut and processed for presentation to mucosal T cells. Regulatory T cells become activated and migrate through the blood and lymph system. Upon encountering antigen in the

target organ, these regulatory T cells release cytokines which suppress or down-regulate autoreactive T cells, thereby ameliorating the disease. By selection of the amount and form of orally delivered protein(s), the suppression is directed toward the tissue under attack in an autoimmune disease.

16. Because the proteins used in the treatment of the invention are naturally derived and subject to normal digestive processes, no toxic effect from their administration is expected, and none has been observed. Their administered dose is typically within the range of edible protein mass (20 mcg to 1 gram/day). The processing of the protein material to reach finished dosage form does not concentrate native impurities or processing residues.

17. Oral tolerance therapy according to the invention utilizes the natural immune system mechanisms associated with the small intestine. These natural mechanisms allow the body to accept nutrition, in the form of foreign proteins, by actively suppressing the immune response that would otherwise mount an attack against such exogenous proteins. The lymphoid tissue of the gut consists of: lymphoid nodules termed Peyer's patches; villi containing epithelial cells; intraepithelial lymphocytes; and lymphocytes scattered throughout the lamina propria, the connective tissue beneath the surface epithelium. Peyer's patches are well-organized lymphoid nodules containing T and B lymphocytes, macrophages, dendritic cells and a germinal center containing B lymphocytes. The nodules appear overlaid by M cells which function to permit antigen uptake and transfer. T lymphocytes present in the dome area of the Peyer's patch are predominantly CD4+ cells of both reactive/inflammatory (Th1) and regulatory (Th2 and

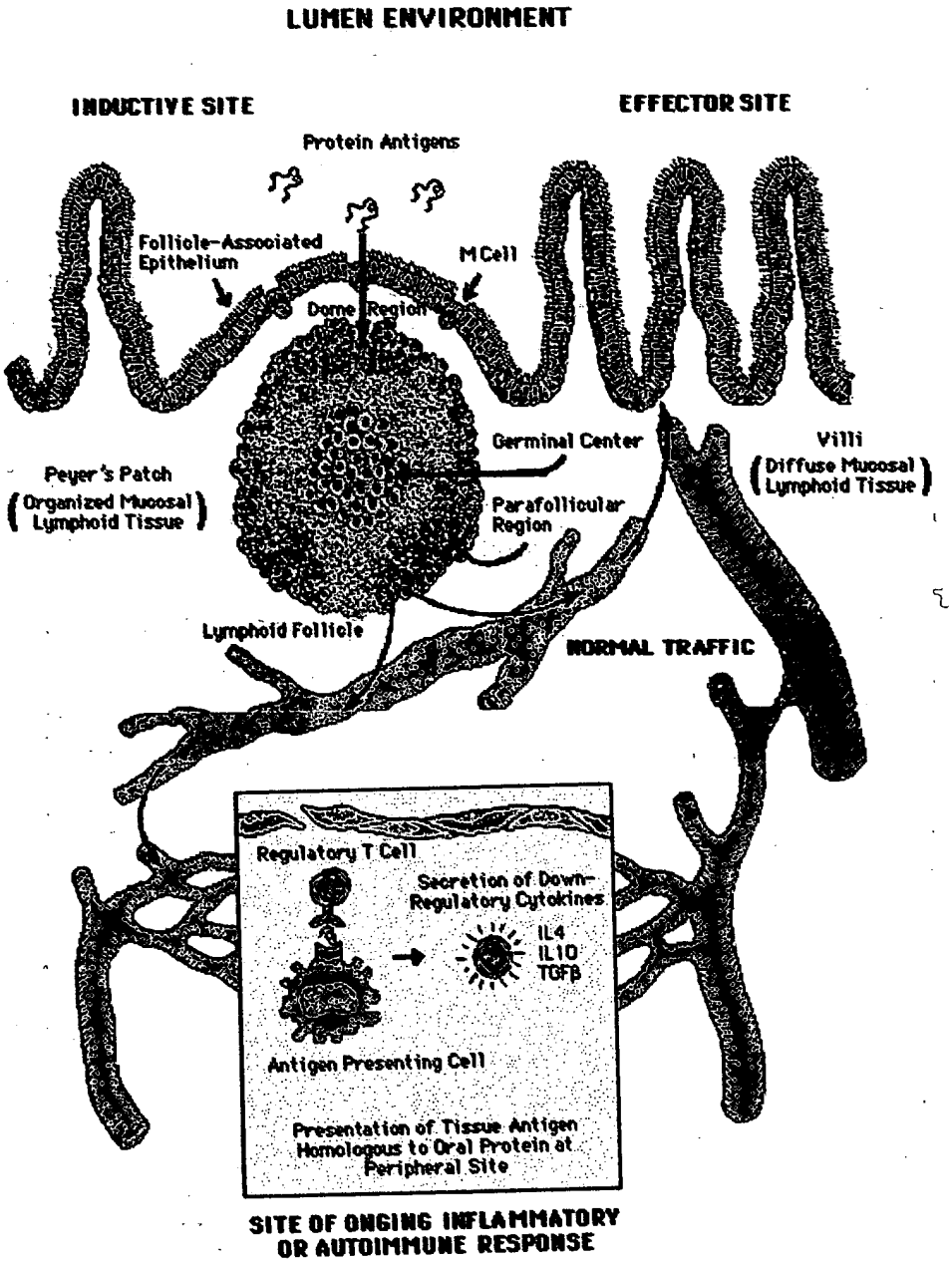
TGF-beta producing) T cell phenotypes, whereas parafollicular T cells (surrounding the lymphoid follicle) are both CD4+ and CD8+6,7. Peyer's patches are a major source of IgA producing B cells. The T cells from the Peyer's patches also have been reported to preferentially induce secretory IgA producing B cells that then differentiate into plasma cells.

18. Orally administered protein exists in several different states as it moves through the digestive system. It can remain intact, be digested into peptide fragments of various lengths, or be degraded into amino acid residues. These molecules enter the gut associated lymphoid tissue either through M cells in the dome of Peyer's patches or through specialized epithelial cells that line the intestinal villi. Small peptide fragments of the protein molecule (generally 9 to 15 amino acids) are presented on the surface of antigen-presenting cells which stimulate B and T cells that are responsive to that peptide. These primed cells then enter the circulation from the vicinity of the Peyer's patches (commonly called inductive regions), migrate through the peripheral circulation and eventually come to settle into other sites in the mucosa (known as effector regions).

19. At least some of the T cells that are stimulated in this fashion can be induced to act as regulatory T cells. Such cells, when subsequently stimulated by the same peptide presented in the major histocompatibility complex (MHC) at the site of local disease, release suppressor cytokines such as IL-4, IL-10 and TGF-beta. These suppressor cytokines decrease inflammatory reactions caused by inflammatory cytokines, such as IL-2, IL-12, gamma-IFN and TNF-alpha, commonly found at sites of active autoimmune disease.

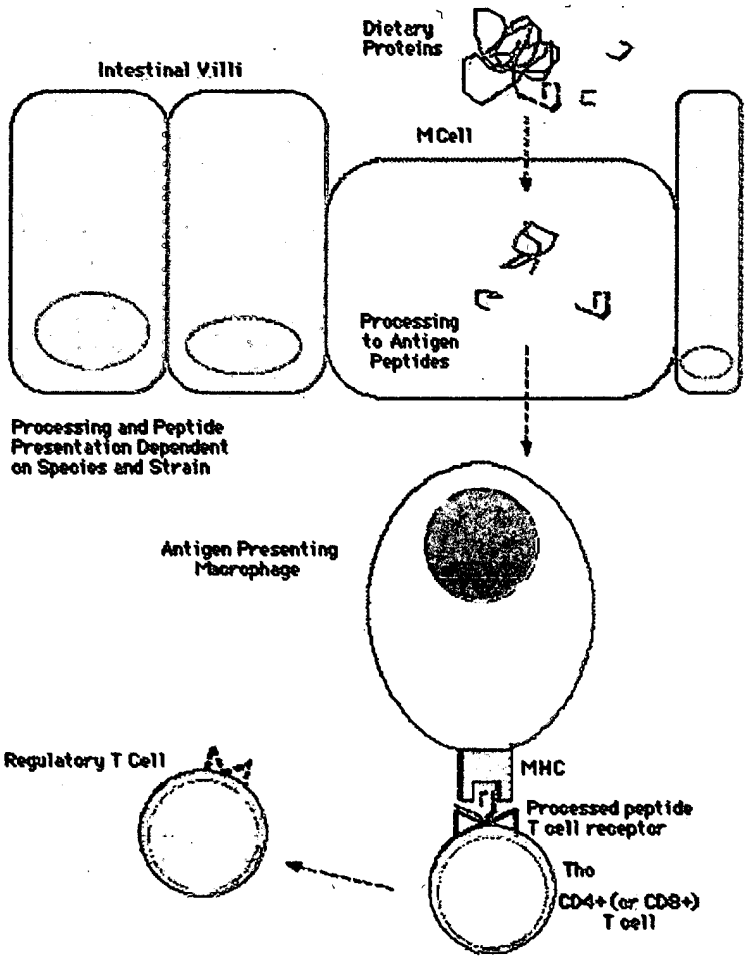
20. A diagrammatic representation of the pathway described above (Figure 1) shows the following sequence of events: entry of protein into the intestinal tract; passage of protein or peptides through gut epithelium; induction of regulatory T cells; transit of the regulatory T cells into the blood stream and lymphatics; and eventual deposition of reactive cells at the site of autoimmune reaction or within the mucosal epithelium.

FIG. 1



21. Presentation of antigens into the gut leads to immunization and oral tolerance. Induction of the IgA antibody response ("immunization") and the induction of regulatory T cells capable of secreting cytokines ("active suppression or oral tolerance induction") are both aspects of the entire mucosal immune response. Figure 2 illustrates protein processing by an M cell and peptide presentation to a T cell in the context of the MHC on a gut-associated antigen-presenting cell.

FIG. 2



22. For active suppression of autoimmune response, antigen presented by gut associated antigen presenting cells preferentially induce regulatory T cells. Regulatory T cells specific for orally administered antigen migrate out of the gut to the lymphoid organs and into the general circulatory system. Upon encountering and recognizing the same or similar antigen in the target (diseased) tissue, the regulatory T cells are stimulated to secrete suppressive cytokines, such as TGF-beta, IL-4 and IL-10. These suppressive cytokines, in turn, function to down regulate the activated inflammatory Th1 cells. Active suppression has been shown to be a primary mechanism of oral tolerance in autoimmune disease models. Regulatory cells have been identified that act via the secretion of antigen-nonspecific down regulatory cytokines (TGF-beta, IL-4, IL-10) when triggered by an oral antigen found in the diseased tissue. Regulatory cells can be found in Peyer's patches 24-48 hours after a single feeding of an antigen such as myelin basic protein (MBP). The antigens do not need to attain access to the circulatory system or organs other than the gut associated lymphoid tissue in order to induce the regulatory cells involved in the suppression of the target autoimmune disease. Circulating blood levels of antigen are not required to induce active suppression.

23. Thus, the present invention employs oral tolerance, a natural immunological process, in the treatment of T-cell mediated autoimmune disease. The overall schemata of oral tolerance as described above has been found to be widely applicable, even broader than the use of autoantigens to treat T-cell mediated autoimmune disease, i.e., even broader than the invention of this application. This mode of treatment has sufficient breadth to

have resulted in initiation of a substantial number of clinical trials, directed to treatment of five diseases. This is, I believe, an exceptional amount of clinical development devoted to a single mode of treatment (oral tolerance), and a strong indication of the breadth of the present invention.

24. For these reasons, I disagree with the statement by the Examiner at the PTO that the invention of this application cannot be practiced generally for the treatment of T-cell mediated autoimmune disease.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

9/9/97
Date

Howard L. Weiner, M.D.

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